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Actinoporins from the sea anemones, tropical *Radianthus macrodactylus* and northern *Oulactis orientalis*: Comparative analysis of structure–function relationships

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ABSTRACT

Actinoporins Or-A and Or-G from the northern sea anemone *Oulactis orientalis* and actinoporins RTX-A and RTX-SII from the tropical sea anemone *Radianthus macrodactylus* (=*Heteractis crispa*) were compared with each other and with some known actinoporins. In this work the complete amino acid sequence of RTX-SII was determined by molecular biology methods. The following differences were revealed in functionally significant regions of *Radianthus*, *Oulactis*, and some other actinoporins: (i) tryptophan is substituted for leucine in the position equivalent to Trp112 in the POC binding site of EqtII; (ii) 13 and 5 residues are truncated in N-terminal regions of Or-A and Or-G, respectively. A possible role of these structural differences in specific regions of the actinoporin sequence was analyzed. Some differences in hydrophobicity parameters, distribution of charged residues, and length of actinoporins' N-terminus apparently cause considerable differences in their hemolytic activities. Homology models of *Radianthus* and *Oulactis* actinoporin monomers were generated using crystal structures of equinatoxin II from *Actinia equina* and sticholysin II from *Stichodactyla helianthus* as templates. The current data on actinoporin structures and activities, coupled with results of our earlier differential scanning calorimetric and electrophoretic experiments with RTX-A-modified erythrocyte ghosts (Shnyrov et al., 1992), suggests that the exposed RGD motif located near the POC binding site can interact with membrane integrin(s).

1. Introduction

Actinoporins are ~20 kDa eukaryotic cytolysins produced by sea anemones and belong to the unique family of α-pore-forming toxins (PFTs) (Anderluh and Maček, 2002). In the course of evolution they probably appeared as killing agents for target cell membranes similar to some bacterial α-PFTs such as colicins from *Escherichia coli* (Parker et al., 1990), diphtheria toxin from *Corynebacterium diphtheriae* (Silverman et al., 1994), exotoxin A from *Pseudomonas aeruginosa* (Allured et al., 1986), and β-PFTs such as α-hemolysin from *Staphylococcus aureus* (Song et al., 1996), CytB δ-endotoxin from *Bacillus thuringiensis* (Li et al., 1996), cholesterol-dependent perfringolysin O from *Clostridium perfringens* (Shatursky et al., 1999), and some other bacterial toxins. Pores formed by α-PFTs according to α-helical bundle model, consist of a few molecules and differ from β-PFTs which consist of oligomeric pores containing seven to fifty protomers, arranged as a β-barrel model (Parker and Feil, 2005).

Abbreviations: ALPs, actinoporin-like proteins; SM, sphingomyelin; PFT, pore-forming toxin; POC, phosphocholine.

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PFTs produced by different organisms have tertiary structures that allow these molecules to be secreted in stable, water-soluble forms that are transformed into membrane-bound forms during the process of insertion into the cell membrane (Parker and Feil, 2005). However unlike three-domain structures of some bacterial PFTs, actinoporins have a peculiar single domain structure, that can reside within cellular and model lipid membranes and form cation-selective transmembrane pores. Their potent pore-forming activity was shown to be sphingomyelin-dependent (Bernheimer and Avigad, 1976; Doyle and Kem, 1989; Turk, 1991; Schön et al., 2008). All known members of the actinoporin family consist of 175–179 amino acids. They contain no cysteine residues and have p*Is* $\geq$9.0. At present, about ten complete amino acid sequences of actinoporins obtained mainly from tropical species have been determined (Anderluh and Maček, 2002). Structure–functional relationships of two actinoporins, EqtII from *Actinia equina* (Anderluh et al., 1996, 1999a,b) and StII from *Stichodactyla helianthus* (Blumenthal and Kem, 1983; Lanio et al., 2001; Huerta et al., 2001), have been studied most extensively (Kristan et al., 2007; Schön et al., 2008). Their crystal and solution structures are known (Athanasiadis et al., 2001; Hinds et al., 2002; Mancheño et al., 2003). In spite of their sequence similarity (65–85%), their hemolytic activities differ quantitatively (Turk, 1991; Khoo et al., 1993; Anderluh and Maček, 2002). Previously we determined that human red cell lytic activities of aqueous extracts from some relatively low latitude, boreal species were significantly less than that of tropical species (Klyshko et al., 2003; Kozlovskaya, 1990). One factor which may contribute to these functional differences is the faunal diversity of the environments these animals inhabit. It is known that the number of potential prey and predator species for sea anemones decreases from tropical to temperate waters (Kostina, 1990). The low level of actinoporin hemolytic and toxic activities of the low boreal species collected in the lower shelf zone may be due to the absence of predators (some starfishes and nudibranchiate mollusks of big sizes), which normally live in the warm shoal waters (Ottaway, 1977). Detritus, plankton, and small shellfishes were found to be the main food for the sea anemones of low boreal waters (Van Praët, 1982). This interpretation assumes that sea anemone PFTs have allelopathic effects on other members of the marine community.

Recently we determined the complete amino acid sequences of *Oulactis* and *Radianthus* actinoporins Or-A, Or-G, and RTX-A, as well as a fragment of RTX-SII (Il'ina et al., 2005a,b, 2006; Klyshko et al., 2004a,b). We found that they possess high sequence identity with the known actinoporins from different species. The comparative study of actinoporins from tropical and northern sea anemones was continued in this work. We report the complete amino acid sequence of RTX-SII determined by molecular cloning methods and describe three-dimensional models of *Oulactis* and *Radianthus* actinoporins using comparative modeling methods. Functionally significant portions of *Oulactis* actinoporins (initially isolated from *Oulactis orientalis* obtained from the Sea of Japan) and N-terminus and phosphocholine (POC) binding site portions of *Radianthus* actinoporins (from tropical *Radianthus macrodactylus*) were also analyzed.

2. Materials and methods

2.1. Materials

Tryptone, glycerol, guanidinium thiocyanate, sarcosile, bacto agar, yeast extract Tris-base, EDTA, and phenol were from MP Biomedicals (Germany); IPTG and X-Gal from Fermentas (Lithuania); agarose from BioRad (USA); chloroform and isopropanol from Reagent (Russia). Reagents for PCR: dNTP, Taq DNA polymerase, primers M13F/R and DNA markers (100–1000 + 1500 bp) were obtained from SibEnzyme (Russia). Other primers were purchased from EuroGene (Russia). ABI PRISM BigDye Terminator v2.0 Ready Reaction Cycle Sequencing Kit for ABI 310 GA was from Applied Biosystems (USA).

2.2. Biological materials

Specimens of the sea anemones *R. macrodactylus* and *O. orientalis* were collected in the coral reefs of the Seychelles and in the intertidal zone of Posiet Bay (the Sea of Japan), respectively, during a marine expedition aboard the research vessel "Academik Oparin". Dr. C.D. Grybelniy (Zoological Institute of the Russian Academy of Sciences, Saint-Petersburg, Russia) and Dr. E.E. Kostina (A.V. Zhirmunsky Institute of the Marine Biology of the Far-Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia) confirmed the identity of the species.

2.3. Isolation of RNA and reverse transcription

Total RNA was isolated from the tentacles of the sea anemones by phenol/chloroform extraction method as described (Chomczynski and Sacchi, 1987). The concentration of the RNA isolated was determined by agarose gel electrophoresis. Smart™ Kit (Clontech, USA) was used for preparing of cDNA.

2.4. Polymerase chain reaction (PCR)

PCR was carried out using the gene-specific and standard "cap"-primers (Table 1), Tag-polymerase, buffer for the Tag-polymerase, the solution of deoxyribonucleotides, and *R. macrodactylus* cDNA as a template. The reaction was carried out on a Master cycler personal amplifier (Eppendorf, Germany) at the following condition: denaturation at 94 °C for 3 min, then 25 cycles: denaturation at 94 °C for 20 s, annealing at 58 °C for 20 s, synthesis at 72 °C for 1 min; and 15 min at 72 °C. The reaction products were analyzed by electrophoresis on a 1.5% agarose gel. A nucleotides kit containing DNA lengths from 100 to 1500 bp was used as a standard.

2.5. Cloning, sequencing and nucleotide analysis

The fragments obtained by PCR were ligated into the pTZ57R vector using InsT/Aclone™ (Fermentas Lithuania). *E. coli* DH5α cells were transformed with aliquots of this